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MICROBIAL SYNTHESIS OF SELINIUM NANOCOMPOSITE USING *SACCHAROMYCES CEREVISIAE* AND ITS ANTIMICROBIAL ACTIVITY AGAINST PATHOGENS CAUSING NOSOCOMIAL INFECTION

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Microbial synthesis of nanocomposite is a green chemistry eco-friendly method. It is a branch of nanoscience for biomedical applications that interconnects nanotechnology and microbial biotechnology. It is only recently that microorganisms have been explored as potential biofactories for synthesis of metal nanoparticles and nanocomposites. By this method, the production efficiency of nanoparticles is very high permitting to relatively easy scale-up the process. Biosynthesis of selenium nanocomposites in aerobic conditions by yeast, *Saccharomyces cerevisiae,* is reported in the present study. Treating sodium selenite solutions with *S. cerevisiae* cells and rapid reduction leads to the formation of highly stable selenium nanoparticles in solution. UV spectrum, XRD and SEM analysis of the selenium nanocomposites indicated that they are in the range of size from 30 to 100 nm size. The nanocomposites were further analyzed for antimicrobial activity against a panel of nosocomial infection causing pathogenic bacteria, and they exhibited significant antimicrobial activity.

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1. Introduction

In the area of nanotechnology, the development of techniques for the controlled synthesis of metal nanoparticles of well-defined size, shape and composition is a big challenge. Metal nanoparticles and nanocomposites exhibit unique electronic, magnetic, catalytic and optical properties that are different from those of bulk metals. This could result in interesting new applications that could potentially be utilized in the biomedical sciences and areas such as optics and electronics [1]. Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity [2]. Biological systems provide many examples of specifically tailored, nanostructured molecules with highly optimized properties and characteristics. These biological materials can be used in their native form directly extracted from the living systems, or they can be processed after extraction and modified to their desired form. Thus, the biological material can be seen as a nanophase system in its own right and as the starting point for producing other novel nanophase systems.

Selenium possesses excellent photoelectrical and semiconductor properties which make it extensively used in duplicate, photography, cells and rectifiers [3]. Selenium is also one of essential trace elements in the human body and has great importance in nourishment and medicine

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[4]. It has been reported that the redness selenium nanoparticles has high biological activities and low toxicity [5, 6, 7]. Medical diagnostic field also developed to use the selenium nanoparticle and also studies on the increase efficiency of glutathione peroxidase and thioredosin reductase [8, 9]. Gao et al. [5] demonstrated the antioxidant properties of hollow spherical nanoparticles of selenium. Nano selenium also reported as antioxidant with reduced risk of direct toxicity on cells [10]. The range of 5- 200 nm sizes of selenium nanoparticles played a significant role on the biological activity especially to scavenge free radicals in vitro condition [11]. The synthesis of nanoparticles using microorganisms and plant extracts has been suggested as a possible green alternative to chemical and physical methods. In recent years, many different techniques have been described for the biological synthesis of silver and gold metal nanoparticles. Although many reports have been previously published about the reduction of selenium oxyanions (selenite and selenate) to elemental selenium under aerobic and anaerobic conditions, the capacity of a large number of bacteria to form selenium nanoparticles has yet to be demonstrated [12, 13]. Hence, the present investigation demonstrates the synthesis of selenium nanocomposites using *S. cerevisiae* and the antimicrobial activity of selenium nanoposites reported.

2. Experimental details

2.1. Microorganisms

The strain yeast *S. cerevisiae* (MTCC 36) culture was obtained from Microbial type culture collection (MTCC) Chandigarh. The culture was reviewed in malt yeast (MY) agar with the following composition: (g/l) malt extract 3.0, yeast extract 3.0, peptone 5.0, glucose 10.0, agar 18.0 and made stock culture. Then the culture subsequently inoculated in the MY medium supplemented with traces of sodium selenite (0.05 mM). This culture was further used for selenium nanoparticles synthesis.

2.2. Effect of selenite stress

The effect of selenite on the growth of the S. *cerevisiae* was determined in the presence of 0.5, 1.0, 2.0, 5.0 and 10.0 mM of sodium selenite. Sodium selenite was prepared as 1 M stock solution and sterilized by filtration. 250 ml Erlenmeyer flasks containing 100 ml of MY medium supplemented with respective concentrations of selenite were inoculated with overnight grown yeast culture and incubated at 30 °C at 200 rpm. Growth was measured by the quantification dry weight of biomass [14].

2.3. Synthesis of selenium nanoparticles

The mid log phase culture of stress tolerant culture was grown in MY medium for 24 h at 30° C at 200 rpm in shaker. After incubation the culture was taken and washed with phosphate buffer for three times. The cells were harvested by centrifugation at 4000×g for 10 min and washed twice with sterile phosphate buffer. For the synthesis of selenium nanoparticles sodium selenite (Sigma, USA) was used. Double-distilled deionized water was used for all the experiments. Synthesis of selenium nanoparticle, about 1 g of wet yeast cells taken along with 50 ml of 2 mM sodium selenite in to 250 ml Erlenmeyer flask and the flasks were incubated in a incubator shaker at 30^{o} C for 24 h at 200 rpm.

2.4. Characterization of selenium nanocomposite

UV-Vis spectroscopy measurement of the disrupted cell with reduced selenium nanoparticle was carried out on JASCO dual-beam spectrophotometer (model V-570) operated at a resolution of 1 nm. To find the highest peak, a spectral scanning analysis was carried out by measuring optical density of the content from wavelength 250 to 700 nm.

The powdered samples of sodium selenite nanoparticles were investigated with X-Ray diffraction method. XRD spectra were obtained using a XPERT-PRO diffractometer

(0000000011014281) with the copper anode (40 KV and 30 mA) and scanning from 24° - 85° C at position 2 Θ. Crystalline deposits obtained from the surface of the treated mortar specimens were added to the sample holder. The XRD data was used to calculate Scherrer formula and stated the range of the nanoparticle size was determined.

After incubation period the cells were harvested and washed three time physiological saline and fixation was carried out with Karnovsky fixative. Further dehydration process was done with alcohol, then freeze dried, then the samples were viewed under scanning electron microscope.

2.5. Antibacterial activity

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumonia, Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus subtilis* were obtained from the Bose Hospital, Madurai Tamil Nadu, India. The antibacterial activity of selinium nanocomposite against all the four human pathogenic Gram negative bacteria of *E. coli*, *P. aeruginosa*, *K. pneumonia, S. typhimurium*, and two Gram positive bacteria of *S. aureus* and *B. subtilis* was evaluated by using agar well diffusion method [15, 16]. The bacterial isolates were maintained on nutrient agar slants that contained peptone, 5.0; beef extract, 3.0; yeast extract 5.0; sodium chloride, 5.0; and agar 15.0 g per liter of distilled water. Muller Hinton Agar (MHA) plates were inoculated with 100 µl of standardized inoculum ($1.5x10^8$ CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells or cups of 6 mm size were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. Freshly prepared selinium nanocomposite with different concentration of (100, 200, 400 and 800 µg) was added into the wells. Streptomycin (15 µg/ml) and sterilized distilled water were used as a positive and negative control respectively. The plates thus prepared were left at room temperature for 15 minutes allowing the diffusion of the extract into the agar [17]. After incubation for 24 h at 37° C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the selinium nanocomposite. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm [18]. The mean and standard deviation of the diameter of inhibition zones were calculated.

2.6. Determination of minimum inhibitory concentration (MIC)

MIC of selinium nanocomposite was determined based on a broth micro dilution method in a 96-well microplate [19]. Briefly, pathogenic microorganisms were cultured overnight at 37° C on Mueller–Hinton (MH) broth and adjusted to a final density of 10^8 CFU/ml by 0.5 McFarland standards. The selinium nanocomposite (1 mg/ml) was mixed with sterile distilled water and twofold serial dilutions were made in the concentration range from 7.8 to 1000 µg /ml. In the 96- well plate, each well had 90 µl of MH broth, 10 µl of bacterial inoculum and 10 µl of different concentrations of selinium nanocomposite. The plate was incubated at 37° C for 12 h. After incubation, the bacterial growth was visually inspected and the lowest concentration of selenium nanocomposite at which no observable bacterial growth or turbidity was taken as the MIC value. The experiments were carried out in triplicate.

3. Result and discussion

3.1 Synthesis of Nanocomposite particles

In order to determine the toxicity of sodium selenite to the yeast growth was observed using turbid metric assay with UV spectrometer and the measurement was also notified. The organism was subjected with different concentration (0.5 to 10 mM) of sodium selenite along with MY medium and incubated. The results showed that, the growth were efficiently obtained up to 2 mM concentration and no significant growth observed in other concentrations. Hence 2 mM concentration was selected to expose the yeast culture for synthesis of selenium nanoparticles.

This revealed that up to 2 mM concentration the yeast culture was resistant. Earlier reports also explained the nontoxic effect of selenite to bacteria with the similar concentration [14]. Dungan et al. [20] reported formation of SeO after 28 h during their studies with *Stenotrophomonas maltophilia* in the presence of selenite. Therefore, this kind of organisms can be used in low-cost biological treatment unit for bioremediation selenium laden effluents.

3.2 Evaluation of nanocomposite particles

To determine the time-point of maximum production of selenium nanocomposite, the absorption spectra of the cell free medium were taken using a UV–VIS spectrometer (Shimadzu, UV Pharma spec 1700 with a resolution of 0.72 nm). The yeast culture subjected with 2 mM sodium selenite showed yellow colour initially and periodically noted the colour change in the medium. The colour was changed yellow to dark brownish and finally red in colour. This indicated that the formation of nanoparticles in the aqueous medium according to the earlier report [21, 22]. The spectrum analysis taken randomly for zero hour to every six hours, the 36 hr incubation showed most increase in absorption (Figure 1). The range of absorption is due to formation of different size of nanocomposite.

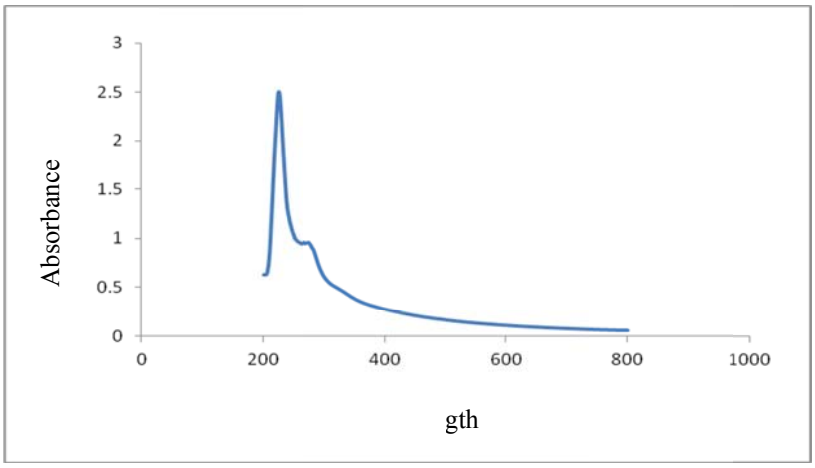

Fig. 1. UV–vis spectra of selenium oxide with S. cerevisiae.

The X-ray diffraction patterns of the samples dried at 30° C and it suggests that the crystalline nature also matched with the standard selenium for confirmation of nanoparticles (Figure 2).

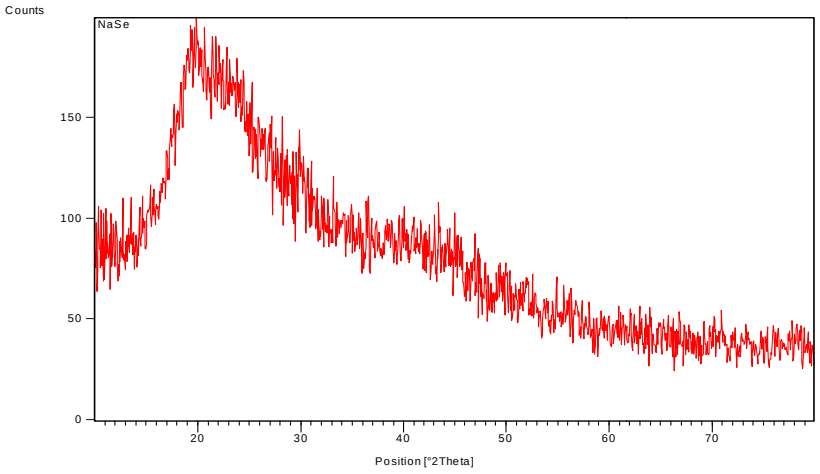

Fig. 2. XRD pattern of the selenium nanocomposite synthesized along with S. cerevisiae.

Earlier reports stated that the selenium nanoparticles crystals size has been studied with the above mentioned formula [21]. Scanning electron microscopy is very important to visualize and get the information about particle size, shape, surface topography, and so forth. Therefore, morphology and structure of the synthesized selenium nanoparticles were also determined by these techniques (Figure 3). The image of the synthesized selenium nanoparticles shows smaller individual particles of about range of 30 - 100 nm size, along with larger agglomerates above the sizes.

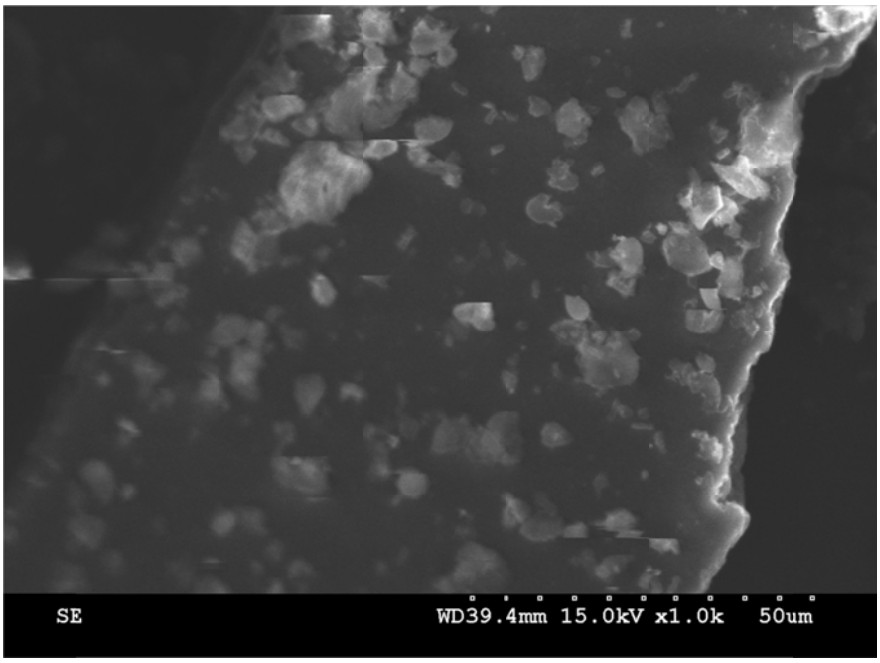

Fig. 3. Image of scanning electron microscopic observation of selenium nanocomposite.

3.3 Antibacterial activity

The antibacterial activity of the selenium nanoparticles were tested against four Gram negative bacterial strains *E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. typhimurium* and two Gram positive strains *S. aureus* and *B. subtilis* (Figure 4). The common antimicrobial agents are extremely irritant and lethal and it is necessary to formulate new types of safe and cost-effective biocidal materials. Studies of Hamouda et al. [23] have shown that antimicrobial formulations in the form of nanoparticles could be used as potential bactericidal materials. A reactive metal nanoparticles display excellent biocidal action against gram-positive and gram-negative bacteria as demonstrated [24, 25]. Accordingly, the preparation, characterization, surface modification, and functionalization of nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials. The highest zones of inhibitions were exhibited against three Gram negative bacterial strains *E. coli*, *P. aeruginosa*, and *S. typhimurium* and one Gram positive strain *S. aureus* [26]. Zhang et al. [27] reported that the antibacterial activity of nanoparticles increased with decreasing particle size and also concentration of nanoparticles increased proportionately.

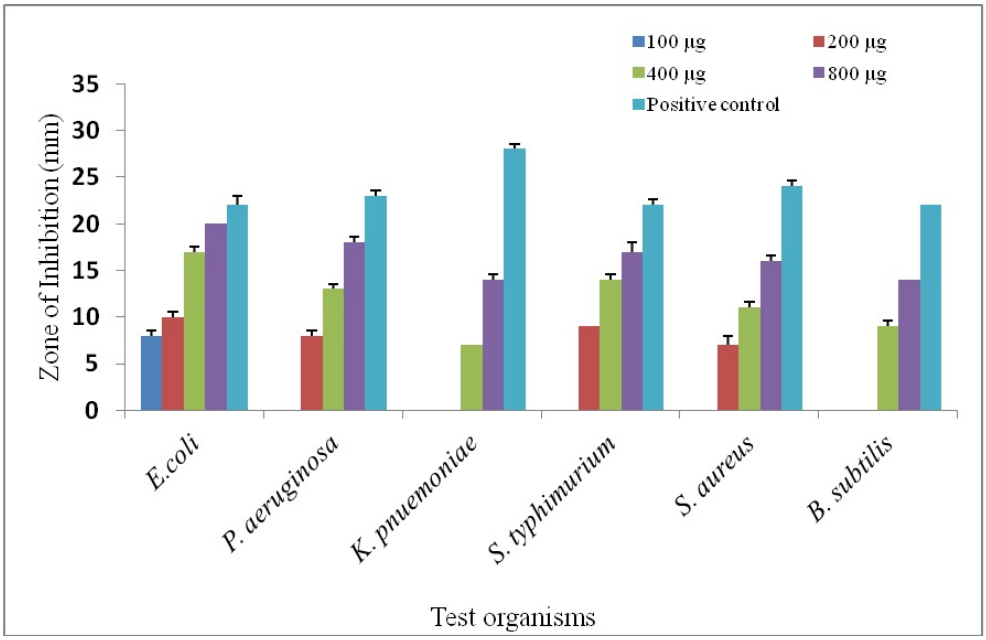

Fig. 4. Antibacterial activity of selenium nanocompostie against nosocomial infection causing bacterial pathogens

3.4 Antibacterial activity of selenium MIC value of selinium nanocomposite

The lowest concentration of selinium nanocomposite at which no growth of microorganisms was observed upon visual observation after incubating at 37° C for 18 h is considered the MIC value. Pellets formed on the bottom of wells were considered bacterial growth even if the wells were clear of turbidity. The lowest MIC value of 31.25 µg/ml *E. coli* S. aureus and the highest MIC value of 250 µg/ml were observed against *K. pneumonia* and *B. subtilis* respectively (Table 1).

Table 1. MIC value of selinium nanocomposite against pathogenic microorganisms.

Test organisms	MIC of selinium nanocomposite (µg/ml)
E.coli	31.25
P. aeruginosa	125
K. pneumonia	250
S. typhimurium	62.5
S. aureus	31.25
B. subtilis	250

4. Conclusion

The potential advantage of microorganisms is the ability to produce nanoparticles and/or nanocomposites in aerobic conditions within short time period and not as much of problem in synthesis and to the environment. *S. cerevisiae* was used for the production of selenium nanocomposites in aerobic culture in limited specification is an advantage and the strain was able to tolerate higher level of dosage. This is an ecofriendly approach and simple step for separation of selenium nanocomposite and treatment also. The use of such nanocomposite for antibacterial activity against virulent pathogens may implicate higher advantage to minimize contamination in hospital environment. Hence, this study gives an approach and practical applications in the field of nanomedicine.

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